ERYTHROCYTE SODIUM FLUXES, INTRACELLULAR MAGNESIUM, CALCIUM AND SERUM ELECTROLYTES IN DIABETICS WITH PREGNANCY-INDUCED HYPERTENSION

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SUMMARY

Serum concentrations of sodium, potassium, ionised calcium, total calcium, magnesium and erythrocyte sodium efflux rate constants were determined spectrophotometrically and with ^{22} Na respectively in normotensive pregnant women (NP), normotensive pregnant women with diabetes mellitus (DP), women with diabetes mellitus and pregnancy-induced hypertension (DP-PIH) and women with pregnancy induced hypertension (NP-PIH) during the third trimester of pregnancy. No significant differences were evident in serum total magnesium sodium, potossium, and ionised calcium or erythrocyte magnesium between the four groups. Serum total calcium, however, was significantly ($p<0.05$) lower in NP-PIH women and PIH women with diabetes mellitus. The cause for the lower serum total calcium in diabetic ahd hon-diabetic PIH women is not evident but appears independent of the diabetes.

Erythrocyte mean total sodium efflux rate constant was significantly ($p < 0.01$) higher in NP-PIH women. However, the mean ouabain-insensitive sodium efflux rate constant was slightly higher in DP women. When compared to the other three groups.

Mean ouabain-sensitive sodium efflux rate constant was significantly ($P< 0.01$; $P < 0.05$) higher in NP-PIH and DP-PIH respectively.

It would appear that while pregnancy-induced hypertension increases the Na+, K+ -AT pase pump activity, this effect is moderated in the presence of a preexisting diabetes mellitus.

INTRODUCTION

The erythrocyte membrane is a highly effective diffusion barrier for cations. It exhibits enough dynamism to changing cellular environment and can therefore serve as a useful model for other more complex somatic cells[1]. For this reason, and because of its ready availability, human erythrocyte membrane serves as a good model for studying membrane phenomenon and enzyme activity in both normal and disease conditions.

Changes in erythrocyte sodium fluxes, intracellular electrolytes and water have been demonstrated in both normotensive and hypertensive patients with either insulin dependent (IDDM) or non-insulin dependent diabetes mellitus (NIDDM) on captopril treatment[2]. Since then other studies have been reported using sodium-22 influx[3] or intracellular electrolytes, such as cytosol calcium[4], as "markers" to reflect the difference between the normotensive, the diabetic and the hypertensive patient. In addition, high blood glucose[5] have been shown to alter erythrocyte functions in diabetes. Similarly changes in calcium and magnesium metabolism have also been observed in pregnancy-induced hypertension (NP-PIH). A reduced serum total calcium with hypocalcuria[6] or hypercalcuria[7] and hypomagnesuria have been reported in mild pregnancy-induced hypertension. The precise mechanism(s) involved in these disturbances is/are not fully understood. It is also uncertain if the pertubations in calcium and magnesium metabolism observed in women with PIH are primary or secondary to the raised blood pressure or to other associated abnormalities. Abnormal or impaired 1,25 (OH)2 D3 production[8] and abnormally elevated PTH[9] have been observed in preeclampsia. These may however be secondary to the

disturbed calcium balance. Very little data however exists on these cations in diabetic women who develop hypertension during pregnancy. It is uncertain if a similar disturbance is also present in some diabetic women who develop hypertension during pregnancy even though considerable evidence exists suggesting an abnormal cellular calcium metabolism in diabetes mellitus[10].This study is therefore designed with the following objectives:-

OBJECTIVES

1. Determine the erythrocyte sodium efflux rate constants in normotensive pregnant women (NP), normotensive pregnant women with diabetes mellitus (DP), women withdiabetes mellitus and pregnancy-induced hypertension (DP-PIH) and women with pregnancy-induced hypertension (NP-PIH).

2. Determine any changes in the serum electrolytes

3. Measure intracellular levels of magnesium and calcium

METHODOLOGY

Experiment 1

1. Determination of serum and intracellular electrolytes

Subjects and sample collection

Participants were 49 volunteers from women attending the antenatal care clinic and consuming an unrestricted diet. Women with a history of hypertension and renal disease or proteinuria prior to the pregnancy were excluded from the study. A written informed consent was obtained and 10 ml of venous blood was collected from an antecubital vein in the third trimester of pregnancy. There were four groups of subjects; normotensive pregnant (NP) (n=10), normotensive diabetic pregnant women (DP) (n=10), pregnancy-induced hypertensives (NP-PIH) (n=20) and diabetcis with pregnancy-induced hypertension (DP-PIH) (n=9). All the diabetic women had IDDM and had been diabetics for at least two years or more. Women with gestational diabetes or evidence of proteinuria prior to the pregnancy were not included.

Blood Pressure Measurement

All blood pressures were measured using a mercury sphygmomanometer. Subjects were seated for about 10 minutes before any measurements were done.

On each occasion three measurements were made over at least five minutes intervals and the lowest reading was recorded. All measurements were made by the same observer. The point of dissappearance of the korotkow sound (K5) was taken as the diastolic pressure. The criteria used for the diagnosis of hypertension was as recommended by the American College of Obstetricians and Gynaecologists were (1) a systolic pressure of > 140 mmHg (2) a diastolic pressure > 85 mmHg (3) an increase of > 30 mmHg in systolic pressure or (4) an increase of > 15 mmHg indiastolic pressure. Any of these criteria were taken to indicate a raised blood pressure when present on at least two occasions separated by at least 6 hours.

Serum analyses

Serum, calcium and magnesium concentrations were determined spectrophotometrically (Micro-flow CL-750). Erythrocyte magnesium concentration was determined colorimetrically (magnesium RBC Biomerieux)- Sodium and potassium concentrations were determined by flame photometery (Corning model 406). Serum ionised calcium was determined using a calcium ion-selective electrode (AVL 984-5). Statistical significance was evaluated by Students t-test with a 'p' < 0.05 considered significant. All values are presented as mean standard error of mean (SEM).

Experiment 2

Determination of sodium efflux rate constants

Venous blood sample (3-4 mls) collected as in experiment 1 was put into a test tube containing heparin and centrifuged at 3000 rpm. The plasma was decanted and the packed cells loaded with a measured amount of radio isotope ^{22} Na and incubated for 2 hours at 37^{o}C. The cells were then washed three times in warm tris buffer of pH 7.4. The cells (1 ml) were then resuspended in 5 ml tris buffer with and without ouabain.

One ml each of these two suspensions was then immediately removed for radioactive counting and estimation of total sample radioactivity. The rest of the two suspensions were then incubated for another 2 hours, while shaking continuously. During this period, timed samples of both suspensions were removed at intervals of 30 minutes up to 2 hours. Each sample taken was immersed in ice and rapidly centrifuged so as to stop active efflux of sodium. The residual radio-activity in the cells was calculated using the formula $-\log_e (1-N_t/N_o)$ where N_t = radioactivity in total sample and N_o = radioactivity in timed sample. The rate of sodium efflux was determined from a plot of residual activity against time. The co-efficient of the regression line was determined for each sample. Regression line(r) with values ranging from 0.999 to 0.960 were used for computing the efflux rate constants. Ouabain was used to separate the ouabain sensitive component from the passive component.

RESULTS

Experiment 1

Age, Gestation and Blood Sugar

No statistically significant differences were evident in the mean ages or in mean periods of gestation between the four groups (Table 1). Random blood sugar levels were significantly higher in women with diabetes mellitus when compared to NP and NP-PIH women ($p < 0.01$). Plasma sugar levels were not significantly different between the two diabetic groups.

Systolic and Diastolic Blood Pressure

Mean systolic and diastolic pressures were significantly higher in diabetic pregnant women (Table 1;$p<0.001$). No significant differences were evident in blood pressures between DP and NP women.

Serum Concentrations of Total and Ionised Calcium

Serum total calcium was significantly lower in DP-PIH and NP-PIH women (Figure 1;$p<0.05$). No significant differences were however evident in mean serum ionised calcium between the four groups (Figure 2).

Serum and Erythrocyte Magnesium Concentrations

Serum total magnesium was not significantly different in the four groups (Figure 3). Similarly, whilst erythrocyte magnesium was slightly lower in DP-PIH women when compared to the other groups. The difference was however not significant statistically (Figure 4).

Serum Concentrations of Sodium and Potassium

No significant differences were evident between the four groups in serum sodium and potassium concentrations (Table 2).

Sodium efflux rate constants

Mean total sodium efflux rate constants of NP-PIH was found to be significantly ($p<0.01$) higher when compared to the values obtained for NP, DP and DP-PIH patients (Fig 5, Table 3).

The mean ouabain sensitive sodium efflux rate contant was significantly ($p<0.01$, $P< 0.05$) higher in NP-PIH and DP-PIH respectively when compared to the other two groups (Fig.6; Table 3).

Mean ouabain insensitive sodium efflux rate constant was only slightly higher in the DP women when compared to the other three groups (Fig 7; Table 3).

	Normotensive pregnant [n=10]	Normotensive pregnant diabetic [n=10]	Diabetic & hyper-tensive [n=9]	Pregnancy induced hypertensive [n=15]
Age (years)	31.4+1.8	32.3+1.5	33.4+2.1	31.3+0.8
Gestation (Weeks)	34.1+1.0	33.0+0.2	34.5+1.2	34.6+0.4
Blood Pressure (mmHg)	114.0+3.2	118.8+4.2	148.0+2.7***	139.0+2.7***
	87.8+3.2	76.8+2.9	96.0+2.4***	92.0+2.4***
Blood sugar	5.5+1.2	9.1+1.0**	9.5+1.2**	5.3+1.2

p<0.01;*p<0.001

Table 1: Mean ages,gestation in Weeks,Blood Pressure & Blood Sugar levels in the four groups

	Normotensive pregnant [n=10]	Normotensive pregnant diabetic [n=10]	Diabetic Pregnant & hyper-tensive [n=9]	Pregnancy induced hypertensive [n=15]
Serum Na^+ $mmol^1$	144.30+0.40	142.60+0.73	144.50+1.09	142.50+1.10
Serum K^+ $mmol^1$	4.05+0.10	4.21+0.08	4.57+0.22	4.47+0.21

Table 2: Serum concentrations of sodium and potassium

Figure 1 Serum total calcium concentration in the four groups

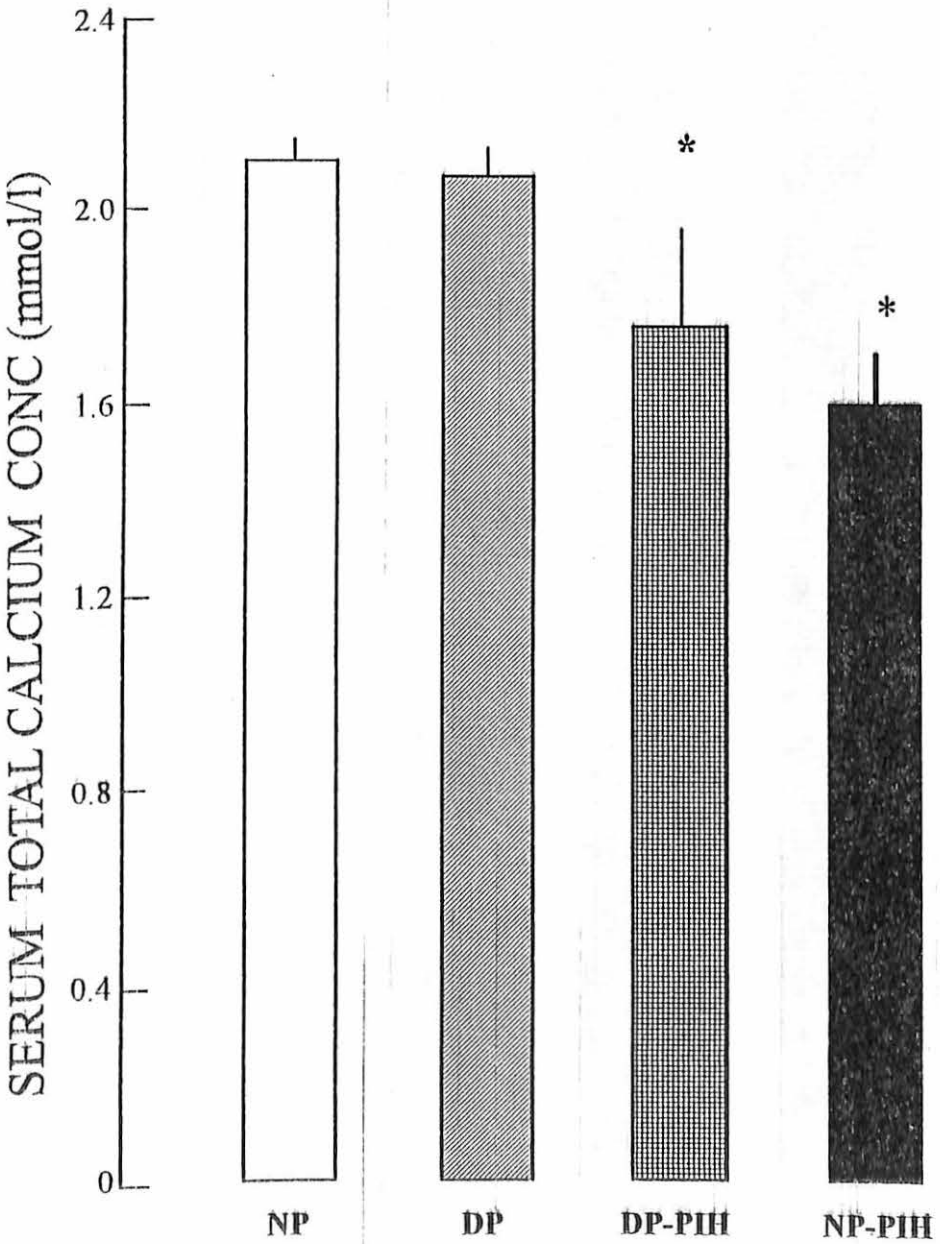


Figure 2 Serum ionised calcium concentration in the four groups

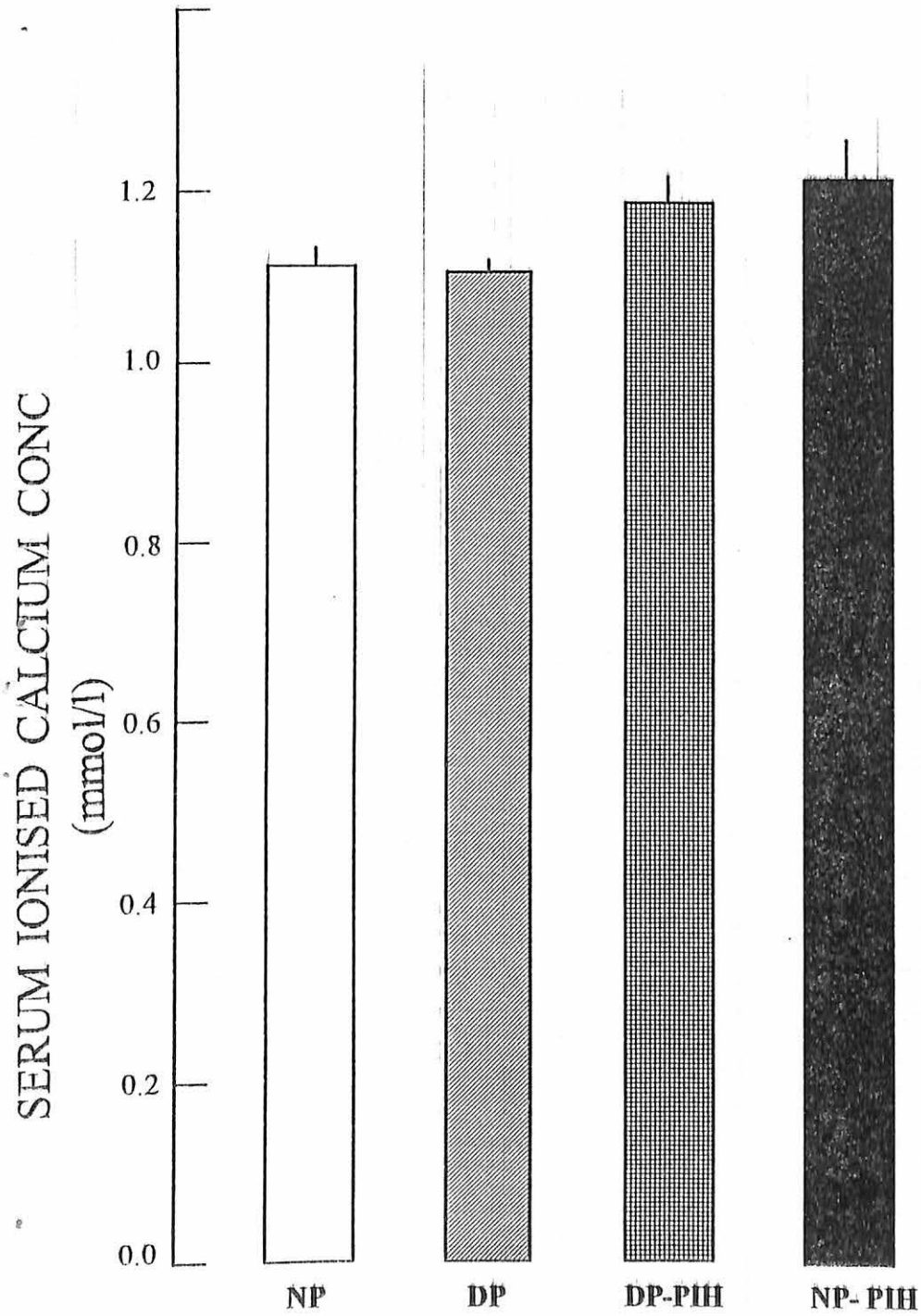


Figure 3 Serum magnesium concentration in the four groups

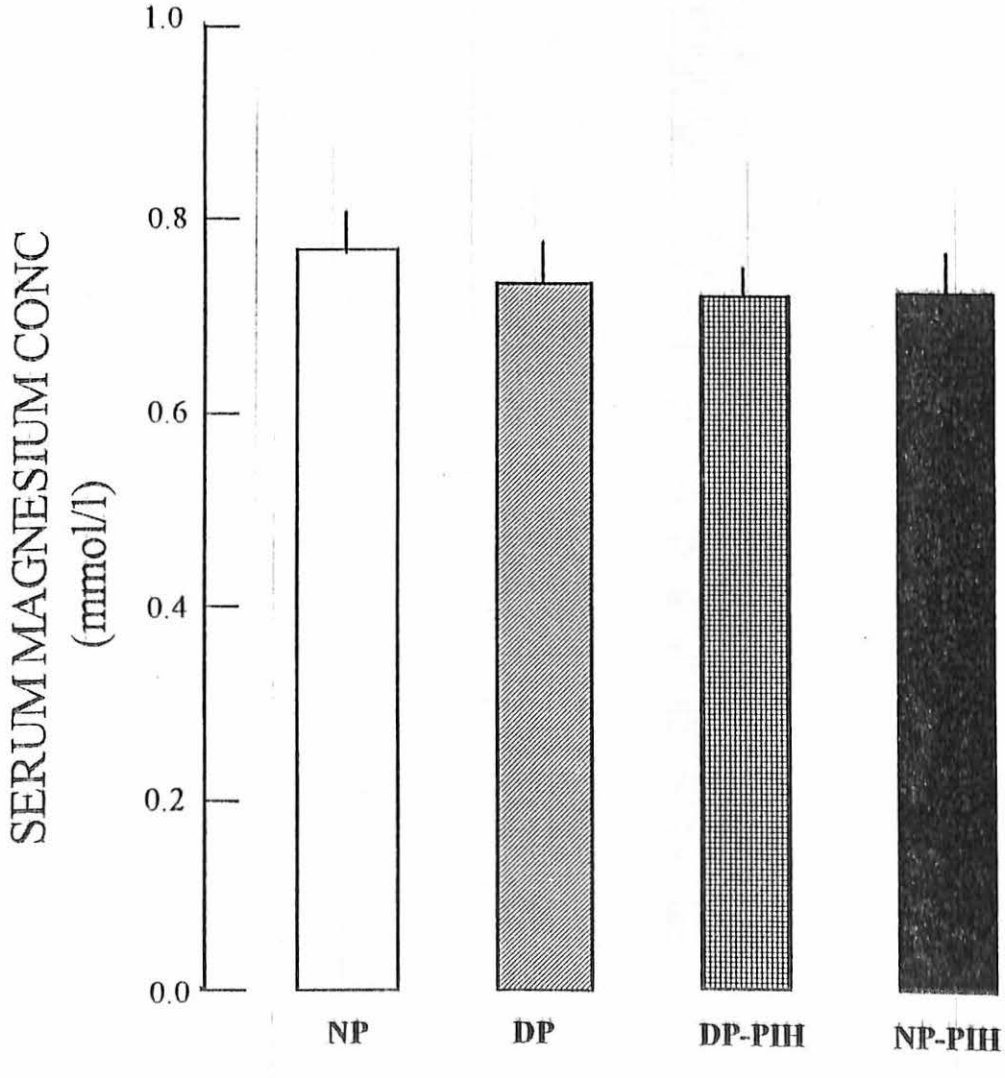
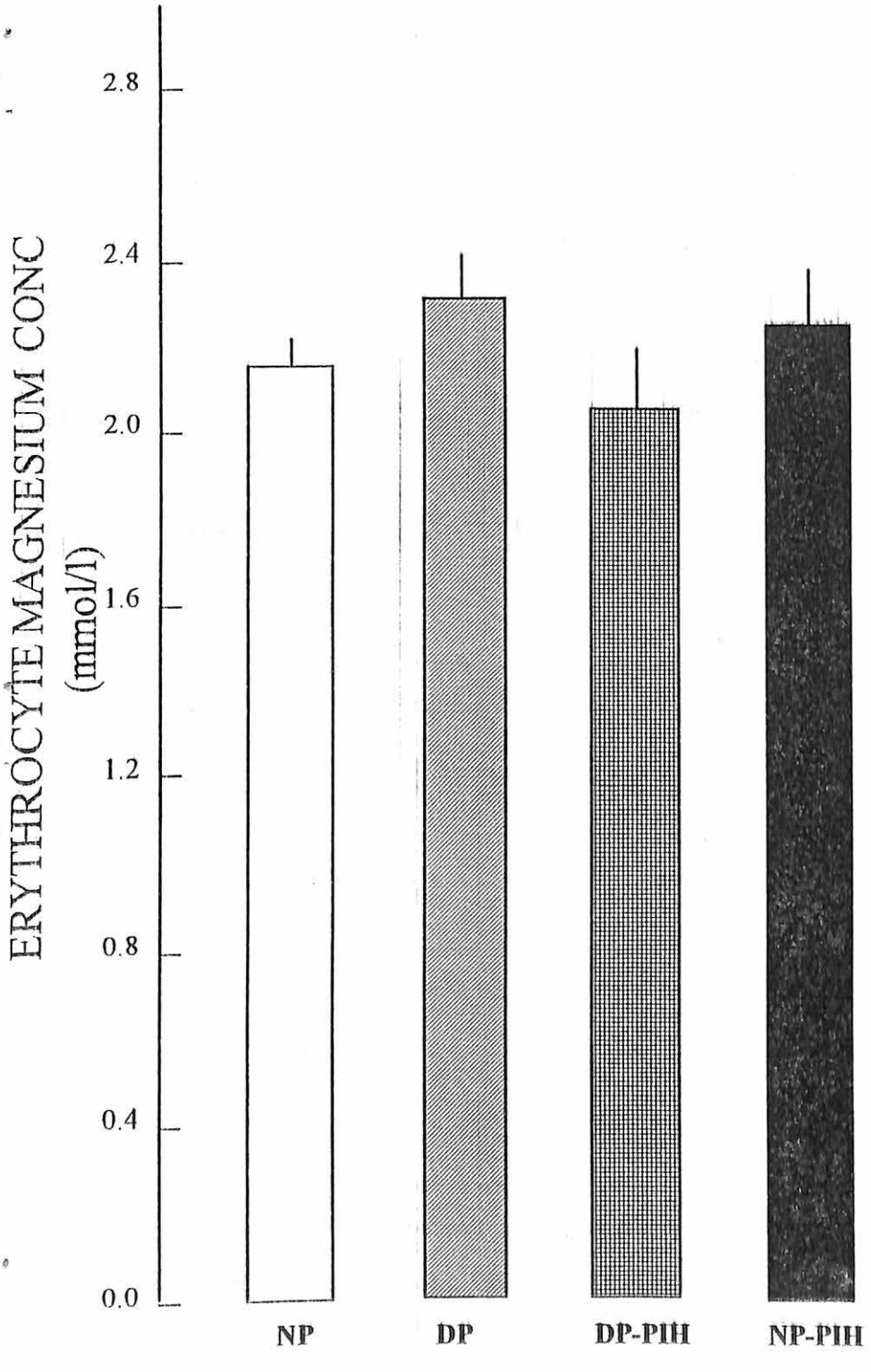


Figure 4 Erythrocyte magnesium concentration in the four groups



Subject	Mean total efflux rate constants (ERC_T) x 10^{-3} min^{-1}	Mean Ouabain-insensitive efflux rate constants (ERC_{01}) x 10^{-3} min^{-1}	Mean Ouabain sensitive efflux rate constants (ERC_{os}) x 10^{-3} min^{-1}
Normotensive pregnant (NP ; n=10)	3.76 ± 0.43	3.35 ± 0.43	0.41 ± 0.19
Normotensive pregnant with PIH (NP-PIH ; n = 10	5.80 ± 0.39**	3.30 ± 0.32	2.5 ± 0.14**
Diabetic pregnant (DP ; n=10)	4.24 ± 0.40	3.79 ± 0.50	0.27 ± 0.15
Diabetic pregnant with PIH (DP-PIH ; n=9)	4.04 ± 0.27	3.21 ± 0.41	0.83 ± 0.22*

Table 3 Erythrocyte mean total, ouabain insensitive, ouabain sensitive sodium efflux rate constants hr^{-1} * $p<0.05$; ** $p<0.01$

Figure 5 Mean total sodium efflux constants in the four groups (p<0.01)**

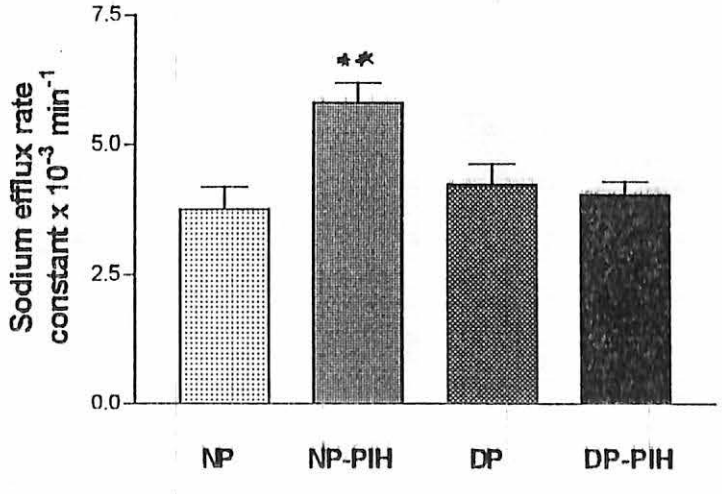


Figure 6 Mean Ouabain insensitive sodium efflux constants in the four groups

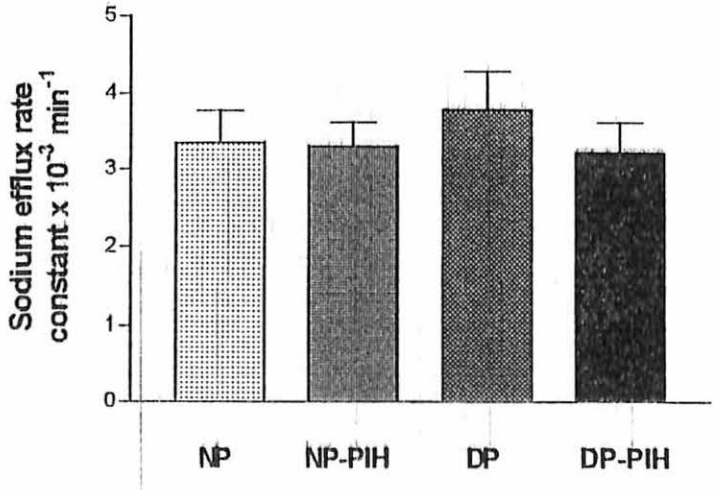
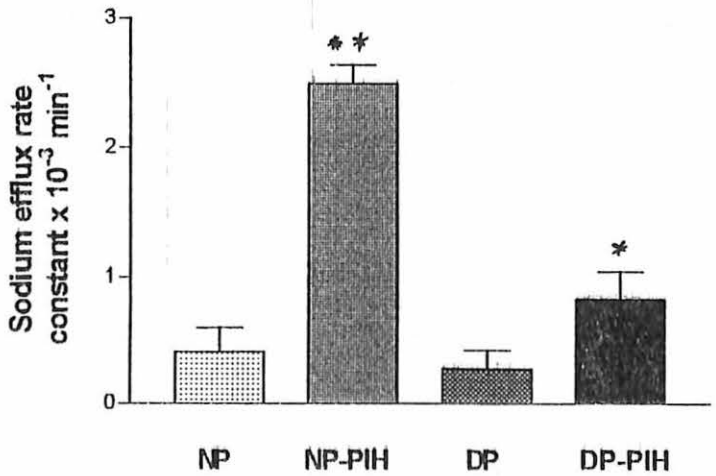


Figure 7 Mean Ouabain sensitive sodium efflux constants in the four groups(*p<0.05;p<0.01)**



DISCUSSION

Systemic arterial pressure was significantly higher in diabetic women with PIH (Table 1). The exact cause for the rise in pressure during gestation is not known and the role of diabetes in this disease entity is also not fully understood. Cardiac output increases whereas total peripheral resistance, as indicated by a fall or unaltered systemic blood pressure, falls during normal gestation. The increase in blood pressure evident in some pregnancies may therefore indicate an inappropriate peripheral vascular resistance. In this respect, pre-eclamptic patients have been shown to have a low pregnancy-associated refractoriness to angiotensin II[11]. The cause of this is uncertain. Vascular tone is influenced by a number of factors including calcium and magnesium. Raised intracellular calcium is known to increase smooth muscle tone whereas magnesium, considered as nature antagonist of calcium, decreases vascular tone[12]. A disturbance in the metabolism of either of these two divalent cations can affect vascular tone and thereby the blood pressure. Cellular calcium metabolism is in fact disturbed in diabetes[10]. It is uncertain if the pre-existing calcium disturbance in DP-PIH women would make the serum calcium profile different from that in women with NP-PIH.

Serum total calcium concentration was significantly lower in DP-PIH women and women with NP-PIH (Fig.1;$p<0.05$) when compared to NP and DP women. The reason for the decreased or lower serum total calcium is not apparent. Comparison between serum ionised calcium concentrations of NP women, DP women, DP-PIH women and women with NP-PIH in this study did not reveal any significant differences between the four groups (Fig. 2). Little data exists on serum ionised calcium concentration in women with diabetes and pregnancy-induced hypertension. Significantly lower serum ionised calcium concentration in women with preeclampsia has been reported[9] although[13,14] found

no significant differences in serum ionised calcium concentrations between their normotensive pregnant and pregnancy-induced hypertensive subjects. The reason for the lower serum total calcium concentration in diabetic and non-diabetic women with PIH in this study is uncertain. No assessment of dietary intake of this cation was however made. Although there is some evidence to suggest that raised plasma glucose concentration may itself lower plasma calcium concentration, [15,16], evidence in the literature overwhemingly fails to support this view. It is therefore unlikely that the lowered plasma calcium concentration evident in DP-PIH women is due to hyperglycaemia per se as a similar decrease was not evident in the DP women. Furthermore, lowered serum total calcium was evident in women with NP-PIH alone in this study and has also been reported in nondiabetic women with mild PIH[13] before. The factor responsible for this in women with PIH is not known. The lowered serum total calcium observed in these women therefore may be due to some other factor or mechanism and may not be due directly to raised plasma glucose concentration, suggesting perhaps that the disturbed glucose metabolism does not predispose to the development of hypertension during pregnancy.

Calcium metabolism in the diabetic state is markedly altered from normal. In human diabetes mellitus, calcium excretion in the urine is increased[17], calcium absorption in the intestine is either increased[18], decreased[19], or normal and[20] bone mass is reduced[21]. Similar observations have also been reported in streptozotocin-induced diabetic rats[22, 23]. Besides, intravenous infusion or ingestion of glucose is associated with increased urinary excretion of calcium both in man[24] and rat [25] suggesting that the increased urinary calcium excretion evident in diabetics is secondary to the raised plasma glucose concentration, possibly involving a direct effect of glucose on tubular reabsorption of calcium[26,27]. Urinary calcium excretion was not determined in this study and is therefore not possible to say if there was any hypercalciuria that could help explain the lowered

serum calcium in these women. Besides, if diabetes induced hyperclaciuria was responsible for the lowered plasma calcium in DP-PIH women then a similarly lowered serum total calcium should have been evident in DP women. The presence of a similarly lowered serum total calcium in women with NP-PIH alone further excludes this possibility. Besides hypocalciuria has been reported in non-diabetic women with PIH[13]. The possibility of the hypocalcaemia evident in DPIH women being secondary to the raised blood pressure also seems unlikely as numerous studies report of a blood pressure lowering effect for calcium[28,29] and a reduced incidence of NP-PIH in women given supplemental calcium during pregnancy[30]. These observations also imply that the lowered serum calcium is most likely contributing to the cause of the raised blood pressure rather than a consequence of it. Whilst it is possible that the lowered serum calcium evident in DP-PIH women may partly be secondary to increased urinary loss coupled with fetal demand, there however appears a possibility that some diabetic women, like other nondiabetic women who develop hypertension during pregnancy, also have a disturbed calcium metabolism that predisposes them or makes them susceptible to the development of hypertension during pregnancy. More investigations are clearly necessitated to illucidate the nature of the calcium disturbance in this disease entity.

Precisely how lowered serum calcium concentration increases blood pressure is uncertain. The role of various calcitrophic hormones has been proposed. Serum PTH has been found to be elevated in some women with PIH[9], and its possible involvement in hypertension has also proposed[31] although a vasorelaxant effect has also been ascribed to this hormone[32]. Besides, normal pregnancy is associated with a slight rise in PTH. The role of PTH in the pathogenesis of hypertension pregnancy therefore seems unlikely.

Changes in plasma levels of 1,25 $(OH)_2$ D_3 in PIH and diabetes have not been extensively studied although evidence published recently suggests an impaired or abnormal $1,25(OH)_2D_3$ production in preeclampsia[33]and PIH[8]. Pregnancy or gestation is associated with alterations in calcium demand and perhaps also the calcium regulating hormones. It is therefore possible that these hormonal deviations may also be involved in the pathogenesis of PIH. Further studies are clearly needed to illucidate the role of calcium and its regulating hormones in hypertension.

Serum total and erythrocyte magnesium concentrations were not significantly different between the four groups (Fig. 3&4). These are in agreement with our previous observations[13]. The absence of a significant difference in serum magnesium concentration between these four groups may not necessarily exclude the possible existence of magnesium imbalance in diabetic and non-diabetic women with PIH, as serum magnesium concentration may not be a true indicator of magnesium status[34]. Besides, twenty-four hour urinary excretion of this cation has been shown to be significantly lowered in women with PIH[13].

From the observations so far it appears diabetic women developing hypertension during pregnancy may also have a disturbed calcium metabolism similar to that in women with PIH alone and independant of their diabetes. The lowered calcium may in some way lead to increased peripheral resistance and a raised blood pressure.

Total sodium efflux rate constants (ERC_T), ouabain-insensitive sodium efflux rate constants (ERC_{oi}) and ouabain-sensitive sodium efflux rate constants (ERC_{os}) of sodium transport in erythrocytes obtained from NP, NP-PIH, DP and DP-PIH were measured using ^{22}Na.

In the present study, the ERC_T for NP-PIH was found to be significantly ($P< 0.01$) higher when compared to the values obtained for NP,DP and DP-PIH respectively (Table 3). This indicates an increase in Na^+ - K^+ contransport activity. Similar results have been reported in NP-PIH[35] in which the increase in pump activity was accompanied by an increase in erythrocyte sodium content. However, the intracellular sodium was not determined in this study. The presence of diabetes mellitus in DP-PIH, did not seen to alter significantly the pump characteristics (Table 3)

Ouabain-insensitive sodium efflux rate constant, which is an index of cell permeability was found to be slightly increased in DP when compared to values obtained for the three groups. This is suggestive of an increased passive diffusion of sodium efflux due presumably to an alteration in erythrocyte plasma membrane properties by diabetes mellitus. Similar modifications induced by gestational diabetes have also been reported[36]. In the presence of hypertension, there was no significant alteration in the passive component (Table 3).

Ouabain-sensitive sodium efflux rate constant was however, found to be significantly ($P < 0.01$) higher in NP-PIH as compared to the values obtained for the other three groups (Figures 5). In the presence of diabetes mellitus and hypertension, the ERC_{os} was also significantly ($P < 0.05$) higher when compared to the values obtained for DP and NP. It would seen that the combined effect of diabetes and hypertension affect the net transport of sodium from the cells. This makes the cells less leaky and promote an increase in intracellular sodium in contrast to that of PIH alone.

It is suggested, therefore that even through diabetes mellitus does alter plasma membrane properties of the erythrocyte, this "leaky membrane" is stabilized in some way by the presence of hypertension. Thus preventing a rapid loss of sodium by the erythrocyte. It is speculated that in DP-PIH the ouabain binding sites may be increased